

Electron-morphometric Taxonomy of *Macrosteles striifrons* Anufriev from East Asia*

Part 1. Discriminant Analysis for Male Population

**Yong Jung KWON, Seung Lak AN¹, Sang Jae SUH²,
Eun Yeop HUH and Jong Kyun PARK**

Department of Agricultural Biology,

College of Agriculture, Kyungpook National University, Taegu 702-701, Korea

¹Department of Natural History, National Science Museum, Taejon 305-338, Korea

²Department of Herb & Food Science, Tongkuk Junior College, Chilgok 718-850, Korea

Abstract Some morphometric multivariate analyses were conducted on the basis of either different geographical, seasonal or host populations of *Macrosteles striifrons*, as this pest species has been confused erroneously with *Macrosteles fascifrons* from North America or invalid several synonyms. The analysis conformed the morphometric distinctions at intraspecific level in male, although some groups overlapped partly between them in different conditions.

Key words Morphometrics, numerical taxonomy, discriminant analysis, Homoptera, Cicadellidae, *Macrosteles striifrons*, East Asia

INTRODUCTION

Leafhoppers of the genus *Macrosteles* are generally associated with herbaceous plants and cause damage resulting in discoloration, wilting or deformation of the plant, by sucking plant sap and transmitting plant pathogens such as viruses and mycoplasma-like organisms.

In East Asia, *Macrosteles striifrons* Anufriev, 1968, is one of the common pests of rice, barley, wheat and vegetables (Okada, 1978; Ishihara, 1983; Kuoh, 1983; Kwon, 1988; Wilson & Claridge, 1991). Especially, Kuoh (1983) includes this species as one of the 7 major Auchenorrhyncha injurious to rice in China.

The present species has been extensively confused with several valid or invalid species erroneously by many authors, due to either wide range of the intraspecific variation or superficial similarity within the genus.

For instances, previous East Asiatic records of "*Macrosteles fascifrons* (Stål, 1858)" [nec, =*M. quadrilineatus* (Forbes, 1885) of North America] actually refer to this species. It has also been known as *M. orientalis* Vilbaste, 1968 in Japan, and as *M. plicativus* Dubovsky, 1970 in Central Asia. Nevertheless, both of the names are placed to the junior synonymy of *M. striifrons*.

The biology of *M. striifrons* has been thoroughly investigated especially in respect to pest status and its transmission of mycoplasma-like organisms (cf. Okada, 1978; Wilson & Claridge, 1991).

For classification in the genus *Macrosteles*, the shape of male genitalia and abdominal apodemes

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provide the most useful interspecific characters (Kwon, 1988). However, in case of the intraspecific discrimination of *M. striifrons*, no such qualitative characters are available currently, and even no work revealed the detailed extent of the intraspecific variation in quantitative characters.

Recently, there have been great advances in the application of multivariate statistics to numerical taxonomy. These allow discrimination between given cases when no single or few characters permit identification, assisted by the use of computers which make these analyses of large quantities of data previously unmanageable practical.

In an attempt to find reliable quantitative characters and to clarify the intraspecific relationships between different geographical populations, seasonal and host representatives of *M. striifrons*, a morphometric analysis was undertaken.

MATERIALS & METHODS

The specimens examined in the present survey were based on the collections from the following institutions:

Department of Entomology, The Natural History Museum, London, U.K.
Department of Entomology, B.P. Bishop Museum, Honolulu, Hawaii, U.S.A.
Canadian National Collections, Biosystematics Research Institute, Ottawa, Canada.
Department of Agriculture, Ministry of Agr. & Coop., Bangkok, Thailand.
Department of Biology, Gorky State University, Gorky, Russia.
Institute of Zoology, Academy of Sciences, Leningrad, Russia.
Department of Agricultural Biology, Kyungpook National University, Taegu, Korea.
Department of Zoology, University of Wales, Cardiff, U.K.

A sample of 20 individuals for either local, seasonal or host populations was taken at random from the above appropriate collections, including reared culture during this survey (Fig. 1).

For each individual specimen, the fore wing and three legs from any convenient side for handling were removed and mounted on a small rectangular piece of card board (Luco/3), and pinned directly beneath the specimen. All the characters measured are shown in Fig. 2. A total of 23 separate measurements on each individual were taken electronically (Table 1), with the aid of an image analyzing system in combination with a stereo microscope (Olympus SZH) and camera lucida, interfaced with a digitizer (Summasketch plus) and microcomputer. The image of the character being measured was projected onto the digitizing pad and traced using a stylus. The information was fed into the computer, analyzed using a software package (Autocad v.10), and the appropriate measurement printed out. This computerized electronic measuring system enables not only simple linear measurements to be made, but also the perimeter and area. All the electronic measurements adopted here are presented in 10 unit microns or 10 unit square microns. Then, computations were carried out through the use of the statistical package, SPSS/PC⁺ (v.2.0). The raw data were standardized and fed into the Wilks' lambda stepwise discriminant analysis, and processed to the cluster analysis using the canonical function scores.

RESULTS

1. Analysis for All 8 Different Populations

The first step of the main analysis was carried out for all 8 male populations incorporating both 3 sympatric populations from Korea distinguished by the occurrence time and hosts, and 5 allopatric ones from different countries.

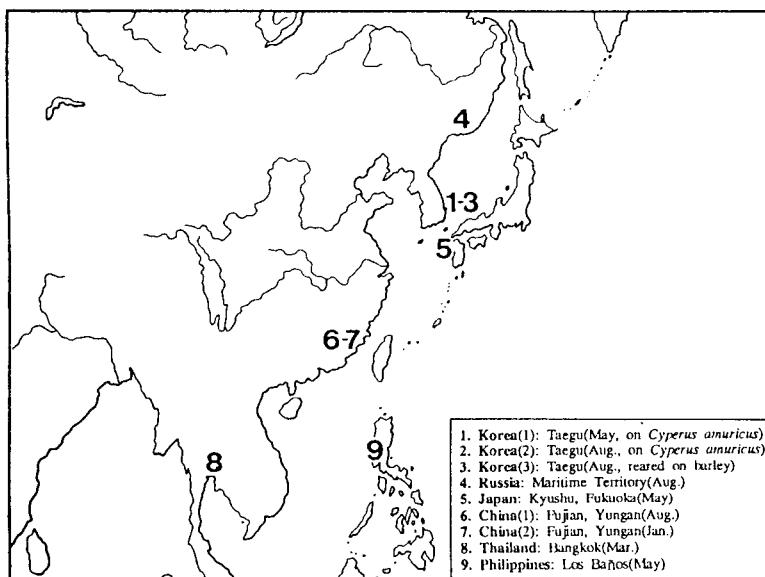


Fig. 1. Localities for the populations of *Macrosteles striifrons* used in the morphometric analysis.

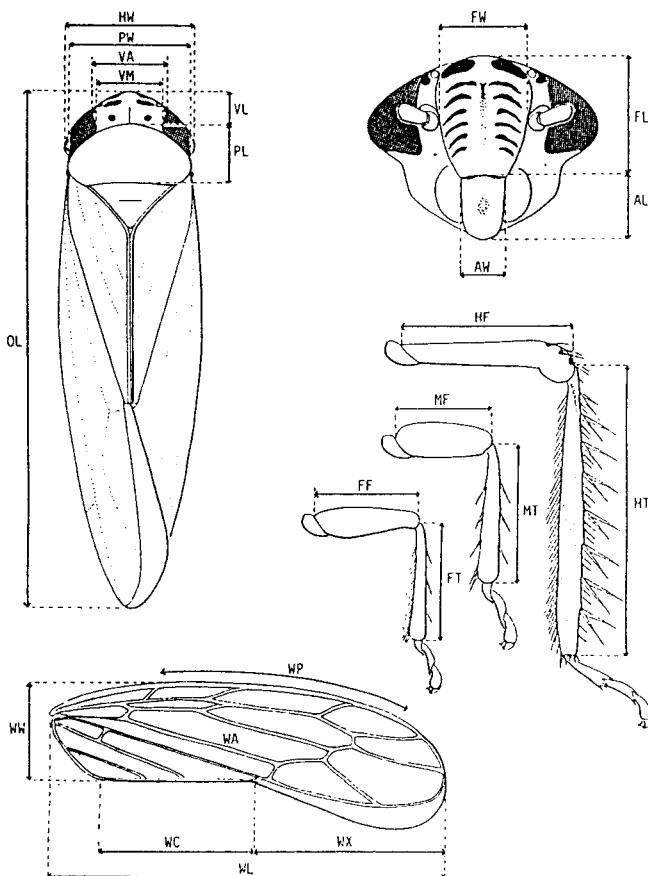


Fig. 2. Schematic drawings of the body parts of *Macrosteles striifrons* (character codes as in Table 1).

Table 1. Codes for characters used in the morphometric analysis of *Macrosteles striifrons*.

Code	Variable
1. OL	Overall length
2. HW	Head width
3. VL	Vertex length
4. VA	Vertex anterior width between eyes
5. VM	Vertex minimum width between eyes
6. FL	Frontoclypeus length
7. FW	Frontoclypeus width between antennae
8. AL	Anteclypeus length
9. AW	Anteclypeus basal width
10. PL	Pronotum length
11. PW	Pronotum width
12. WL	Fore wing length
13. WW	Fore wing width
14. WA	Fore wing area
15. WP	Fore wing perimeter
16. WC	Fore wing commissure length
17. WX	Fore wing appendix length
18. FF	Fore femur length
19. FT	Fore tibia length
20. MF	Middle femur length
21. MT	Middle tibia length
22. HF	Hind femur length
23. HT	Hind tibia length

The purpose of this analysis is to reveal how the selected 3 sympatric Korean groups segregated between themselves, when all 8 groups were analyzed together to compare the morphometrical variation in each population.

As the result, 7 canonical discriminant functions were derived in the analysis. Although the first 4 functions were statistically very significant having an accumulative variance of 94.01% ($p<0.001$), the group centroids were plotted 3-dimensionally against function 1, 2 and 3, as they had high variances and large ratios between groups to within groups sum of squares, with the accumulative score of 87.73% (Fig. 3).

Tests of significances between pairs of group centroids using F-statistics were carried out after step 22. The 3 Korean groups ($p>0.01$) differed insignificantly from the other populations (Table 2). Smaller Wilks' lambda value and larger univariate F-ratio revealed that the perimeter of the forewing was the most powerful discriminator, and all the characters were significant ($p<0.001$) to the discrimination (Table 3).

Predicted group membership gave an average of 83.13% of individuals classified to their known group. Only group 7 (Thailand) revealed 100% successful classification, on the other hand, group 2 (Korea 2) was 60% correct, with 35% predicted for group 3 (Korea 3) and 5% for group 4 (Russian Far East). The intraspecific morphometrical distinction, as a rule, was less significant between the 3 sympatric Korean groups than in case of the allopatric ones from different countries (Table 4).

2. Analysis for 6 Geographic Groups

To evaluate any morphometrical distinction in different countries, the 3 sympatric Korean popula-

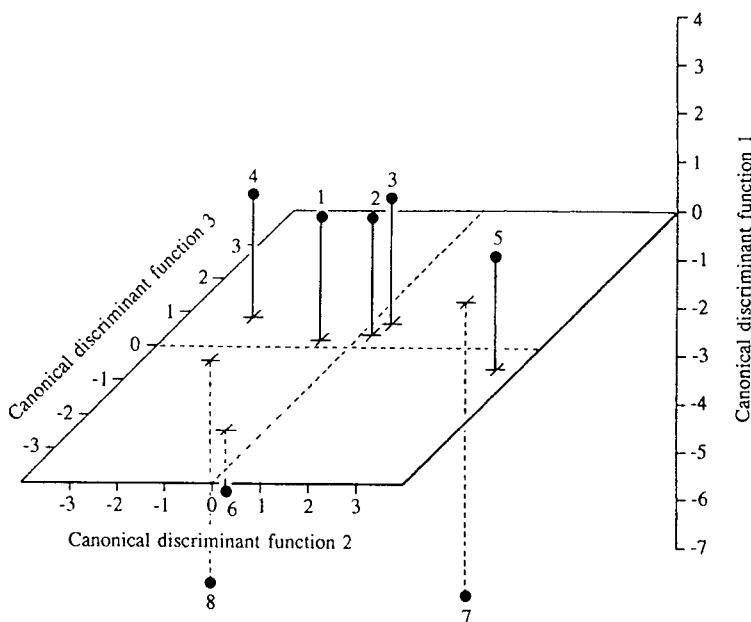


Fig. 3. 3-dimensional plot of the first 3 canonical discriminant functions by group centroids for all 8 populations in male.

Table 2. F-statistics and significances between pairs of all 8 populations in male after step 22 (each F-statistic has 22 and 131 degrees of freedom).

Group	1	2	3	4	5	6	7
2 Korea 2	2.198 .003						
3 Korea 3		2.360 .001	.658 .872				
4 Russia			4.848 .000	6.728 .000	5.955 .000		
5 Japan				10.307 .000	7.880 .000	6.901 .000	16.293 .000
6 China 2					8.790 .000	9.119 .000	10.239 .000
7 Thailand						12.265 .000	15.378 .000
8 Philippines							31.201 .000
							29.552 .000
							29.758 .000
							35.784 .000
							31.667 .000
							16.674 .000
							22.358 .000
							21.993 .000
							22.532 .000
							21.979 .000
							28.646 .000
							9.560 .000
							8.674 .000

tions were merged into the single group so as to compare 6 different allopatric groups.

As the result, 5 canonical discriminant functions were derived, and all were statistically very significant ($p < 0.001$). This was confirmed by the F-statistics and significance tests between pairs of group centroids after step 22 (Table 5).

The first 3 functions revealed an accumulative variance of 90.18%, and the group centroids were plotted 3-dimensionally (Fig. 4).

As in the previous analysis, all the characters entered were significant ($p < 0.001$), and the most

Table 3. Tests for univariate equality of group means for all 8 populations in male.

Variable	Wilks' Lambda	F	Sign.	Variable	Wilks' Lambda	F	Sign.
OL	.25834	62.3	.0000	WW	.19183	91.4	.0000
HW	.43777	27.8	.0000	WA	.23565	70.4	.0000
VL	.75967	6.8	.0000	WP	.14119	132.1	.0000
VA	.42879	28.9	.0000	WC	.21251	80.4	.0000
VM	.36776	37.3	.0000	WX	.18416	96.2	.0000
FL	.42727	29.1	.0000	FF	.25770	62.5	.0000
FW	.50329	21.4	.0000	FT	.19084	92.0	.0000
AL	.77007	6.4	.0000	MF	.34270	41.6	.0000
AW	.64085	12.1	.0000	MT	.22353	75.4	.0000
PL	.42297	29.6	.0000	HF	.34219	41.7	.0000
PW	.25870	62.2	.0000	HT	.24890	65.5	.0000
WL	.16324	111.3	.0000				

Table 4. Predicted group membership for all 8 populations in male.

Actual group	No. of cases	Predicted group membership							
		1	2	3	4	5	6	7	8
1 Korea 1	20	15	3	1	0	0	1	0	0
		75%	15%	5%	0%	0%	5%	0%	0%
2 Korea 2	20	0	12	7	1	0	0	0	0
		0%	60%	35%	5%	0%	0%	0%	0%
3 Korea 3	20	1	5	14	0	0	0	0	0
		5%	25%	70%	0%	0%	0%	0%	0%
4 Russia	20	0	0	2	18	0	0	0	0
		0%	0%	10%	90%	0%	0%	0%	0%
5 Japan	20	0	0	2	0	18	0	0	0
		0%	0%	10%	0%	90%	0%	0%	0%
6 China 2	20	0	1	0	0	0	18	0	1
		0%	5%	0%	0%	0%	90%	0%	5%
7 Thailand	20	0	0	0	0	0	0	20	0
		0%	0%	0%	0%	0%	0%	100%	0%
8 Philippines	20	0	0	0	0	0	0	2	18
		0%	0%	0%	0%	0%	0%	10%	90%

Percent of "grouped" cases correctly classified: 83.13%

Table 5. F-statistics and significances between pairs of 6 geographic groups in male after step 22 (each F-statistic has 22 and 133 degrees of freedom).

Group	1	2	3	4	5
2 Russia	7.736 .000				
3 Japan	11.469 .000	15.791 .000			
4 China	13.225 .000	12.300 .000	14.893 .000		
5 Thailand	45.004 .000	35.934 .000	32.025 .000	16.669 .000	
6 Philippines	33.045 .000	22.149 .000	28.692 .000	9.656 .000	8.721 .000

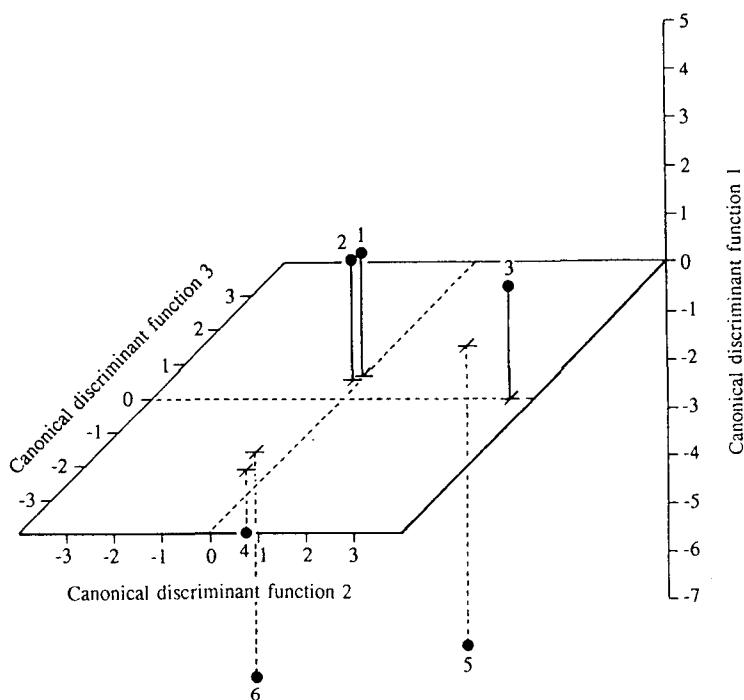


Fig. 4. 3-dimensional plot of the first 3 canonical discriminant functions by group centroids for 6 geographic groups in male.

Table 6. Tests for univariate equality of group means for 6 geographic groups in male.

Variable	Wilks' Lambda	F	Sign.	Variable	Wilks' Lambda	F	Sign.
OL	.25901	88.1	.0000	WW	.19361	128.3	.0000
HW	.44138	38.9	.0000	WA	.23744	98.9	.0000
VL	.76040	9.7	.0000	WP	.14590	180.3	.0000
VA	.42932	40.9	.0000	WC	.21361	113.4	.0000
VM	.37321	1.7	.0000	WX	.19138	130.1	.0000
FL	.43317	40.3	.0000	FF	.26248	86.5	.0000
FW	.50928	9.6	.0000	FT	.19092	130.5	.0000
AL	.78658	8.3	.0000	MF	.35413	56.1	.0000
AW	.64577	16.8	.0000	MT	.22429	106.5	.0000
PL	.43295	40.3	.0000	HF	.34520	58.4	.0000
PW	.25979	87.7	.0000	HT	.25274	91.0	.0000
WL	.16850	152.0	.0000				

powerful discriminator was the perimeter of the fore wing, whereas the length of the anteclypeus was the least (Table 6).

In the classification results, the average predicted group membership was improved and 93.75% of all individuals were correctly assigned to their known local groups. The group 5 (Thailand) also revealed 100% correct assignment as the previous analysis. Thus, the intraspecific morphometrical distinction in different localities was proved to be recognizable though slightly overlapped in most groups (Table 7).

Table 7. Predicted group membership for 6 geographic groups in male.

Actual group	No. of cases	Predicted group membership					
		1	2	3	4	5	6
1 Korea	60	56 93.3%	2 3.3%	0 0%	2 3.3%	0 0%	0 0%
2 Russia	20	1 5%	19 95%	0 0%	0 0%	0 0%	0 0%
3 Japan	20	1 5%	0 0%	19 95%	0 0%	0 0%	0 0%
4 China	20	1 5%	0 0%	0 0%	18 90%	0 0%	1 5%
5 Thailand	20	0 0%	0 0%	0 0%	0 0%	20 100%	0 0%
6 Philippines	20	0 0%	0 0%	0 0%	0 0%	2 10%	18 90%

Percent of "grouped" cases correctly classified: 93.75%

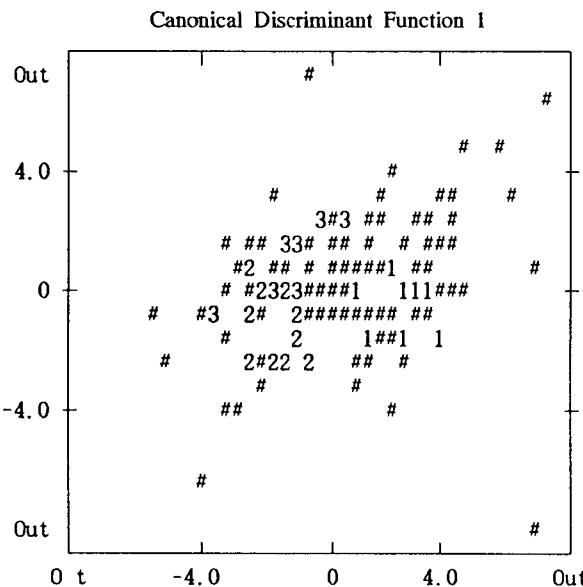


Fig. 5. All-groups scatterplot of canonical discriminant function 1 against function 2 for 3 Korean groups in male (#: ungrouped cases).

3. Analysis for 3 Korean Groups

To find any morphometrical segregation in different seasons and hosts within the same locality, the 3 sympatric populations from Korea were compared alone in this analysis.

Of the 2 canonical discriminant functions derived, the first one was statistically very significant with a variance of 99.61% ($p < 0.001$). The 2 functions were used for the all-groups scatterplot (Fig. 5). Tests of significance between pairs of group centroids using F-statistics were carried out after step 21. The significance between group 1 (spring & *Cyperus*-feeding population) and group 2 (autumn & *Cyperus*-feeding population), representing different season but the same host, was highly apparent ($p < 0.001$). Whereas that ($p > 0.1$) between group 2 and group 3 (autumn & barley-feeding

Table 8. F-statistics and significances between pairs of 3 Korean groups in male after step 21 (each F-statistic has 15 and 43 degrees of freedom).

Group	1	2
2 Korea 2	8.001 .000	
3 Korea 3	6.171 .000	1.204 .304

Table 9. Tests for univariate equality of group means for 3 Korean group in male.

Variable	Wilks' Lambda	F	Sign.	Variable	Wilks' Lambda	F	Sign.
OL	.99340	.189	.8279	WW	.97303	.790	.4587
HW	.95636	1.301	.2803	WA	.97746	.657	.5222
VL	.99729	.077	.9256	WP	.93566	1.960	.1503
VA	.99103	.257	.7736	WC	.98790	.349	.7068
VM	.96241	1.113	.3355	WX	.89978	3.174	.0493
FL	.93629	1.939	.1532	FF	.94727	1.586	.2136
FW	.95556	1.326	.2737	FT	.99909	.026	.9743
AL	.91588	2.618	.0817	MF	.90408	3.024	.0565
AW	.97941	.599	.5527	MT	.98760	.357	.7007
PL	.95074	1.477	.2370	HF	.97602	.700	.5007
PW	.98771	.354	.7029	HT	.96288	1.099	.3403
WL	.92610	2.274	.1122				

population), representing the same season but different host, was insignificant (Table 8).

Although the length of the fore wing appendix was the most powerful discriminator among the characters used, the significance of the others ($p>0.08$) were relatively low, comparing the previous cases (Table 9).

In the classification results, the group 1 (spring & *Cyperus*-feeding population) revealed 95% correct assignment whereas the remainder showed 75% respectively. Overall, 81.67% of all individuals were correctly classified to their own group. Therefore, the intraspecific morphometrical differences were proved to be existent, and more evident by seasons than by hosts within the 3 sympatric populations from Korea (Table 10).

4. Analysis for 2 Different Seasonal Groups from Korea

This analysis was conducted to evaluate the seasonal variation within the same locality. Both the spring and the summer sympatric populations feeding on the same host (*Cyperus*) from Korea were compared separately. The F-statistic between the 2 group centroids after step 24 was highly significant ($p<0.001$). The discrimination achieved between the 2 groups was shown by the stacked histogram which revealed the 2 morphometrically distinct groups (Fig. 6).

As in the previous analysis, the length of the fore wing appendix was proved to be the most powerful discriminator, although the significance ($p>0.2$) was comparatively low (Table 11).

The predicted group membership revealed that 100% of all individuals were successfully classified to their known group. Thus, the 2 sympatric populations feeding on the same host detected to be distinct morphometrically by seasons (Table 12).

Table 10. Predicted group membership for 3 Korean groups in male.

Actual group	No. of cases	Predicted group membership		
		1	2	3
1 Korea 1	20	19 95%	0 0%	1 5%
2 Korea 2	20	0 0%	15 75%	5 25%
3 Korea 3	20	1 5%	4 20%	15 75%
Ungrouped cases	100	55 55%	19 19%	26 26%

Percent of "grouped" cases correctly classified: 81.67%

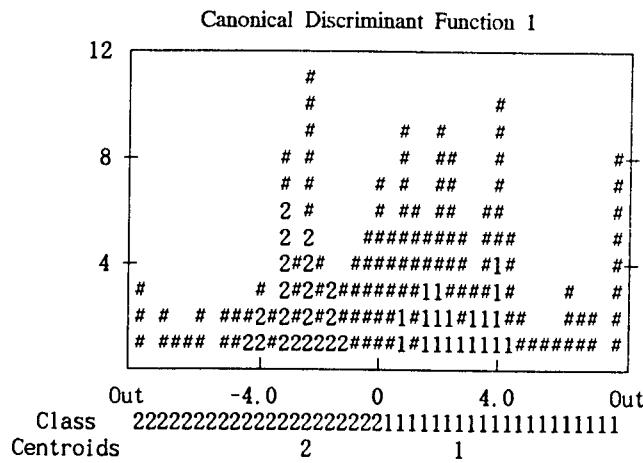


Fig. 6. All-groups stacked histogram of canonical discriminant function 1 for 2 different seasonal groups from Korea in female (#: ungrouped cases).

Table 11. Tests for univariate equality of group means for 2 different seasonal groups from Korea in male.

Variable	Wilks' Lambda	F	Sign.	Variable	Wilks' Lambda	F	Sign.
OL	.99290	.271	.6051	WW	.98069	.748	.3924
HW	.98519	.571	.4545	WA	.96844	1.238	.2728
VL	.99960	.015	.9027	WP	.95442	1.815	.1859
VA	.99381	.236	.6295	WC	.99831	.064	.8010
VM	.99964	.013	.9072	WX	.87625	5.367	.0260
FL	.92417	3.118	.0855	FF	.94684	2.133	.1523
FW	.94523	2.202	.1461	FT	.99880	.045	.8321
AL	.89109	4.644	.0376	MF	.93220	2.764	.1047
AW	.97280	1.063	.3091	MT	.98664	.514	.4775
PL	.94382	2.262	.1409	HF	.98858	.439	.5115
PW	.98144	.718	.4019	HT	.97931	.802	.3759
WL	.88994	4.700	.0365				

Table 12. Predicted group membership for 2 different seasonal groups from Korea in male.

Actual group	No. of cases	Predicted group membership	
		1	2
1 Korea 1	20	20	0
		100%	0%
2 Korea 2	20	0	20
		0%	100%
Ungrouped cases	120	73	47
		60.8%	39.2%

Percent of "grouped" cases correctly classified: 100%

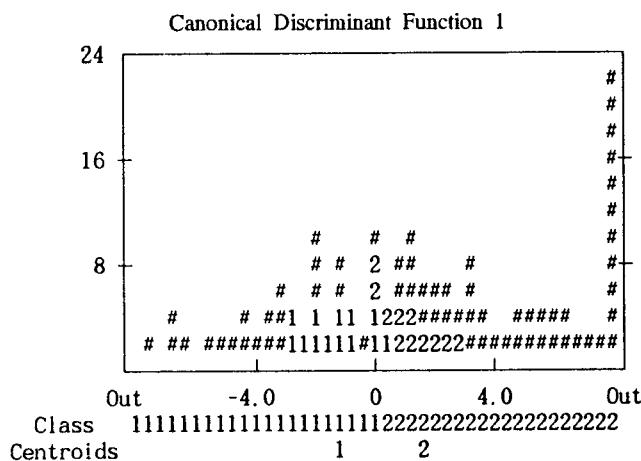


Fig. 7. All-groups stacked histogram of canonical discriminant function 1 for 2 different host groups from Korea in male (#: ungrouped cases).

Table 13. Tests for univariate equality of group means for 2 different host groups from Korea in male.

Variable	Wilks' Lambda	F	Sign.	Variable	Wilks' Lambda	F	Sign.
OL	.99352	.248	.6214	WW	.99671	.125	.7252
HW	.98354	.635	.4302	WA	.99783	.082	.7754
VL	.99661	.129	.7212	WP	.99283	.274	.6035
VA	.98625	.529	.4712	WC	.99205	.304	.5843
VM	.96467	1.392	.2455	WX	.99963	.014	.9064
FL	.97447	.995	.3247	FF	.99879	.046	.8310
FW	.99113	.340	.5633	FT	1.00000	.082	1.0000
AL	.98148	.717	.4024	MF	.98787	.466	.4987
AW	.99890	.041	.8392	MT	.99984	.061	.9378
PL	.99841	.060	.8072	HF	.99245	.289	.5940
PW	.99244	.289	.5937	HT	.99270	.279	.6001
WL	.99160	.322	.5737				

5. Analysis for 2 Different Host Groups from Korea

To evaluate the morphometrical variation by different hosts in the same season and locality, the 2 sympatric Korean populations, feeding on *Cyperus* and barley respectively, were compared separately.

The discrimination between the 2 host groups was shown by the stacked histogram which revealed

Table 14. Predicted group membership for 2 different host groups from Korea in male.

Actual group	No. of cases	Predicted group membership	
		2	3
2 Korea 2	20	15 75%	5 25%
3 Korea 3	20	2 10%	18 90%
Ungrouped cases	120	41 34.2%	79 65.8%

Percent of "grouped" cases correctly classified: 82.5%

a slight overlap between the groups (Fig. 7).

In the U-statistics and univariate F-ratio, most characters used were comparatively insignificant for the discrimination of groups ($p>0.2$), though the minimum width of the vertex between eyes entered firstly (Table 13).

Nevertheless, the F-statistic between the 2 group centroids after step 13 was relatively significant ($p<0.05$). As for the predicted group membership, the *Cyperus*-feeding population (Korea 2) revealed apparently lower assignment than that of the barley-feeding population (Korea 3). Overall, 82.5% of all individuals were correctly classified to their own population. Therefore, the intraspecific variation between the 2 different host populations were recognized, though overlapped in some extent (Table 14).

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東部아시아產 꼭지매미충의 電子計量形態學的 分類*

1. 雄性個體群의 判別分析

권용정 · 안승락¹ · 서상재² · 허은엽 · 박종균

경북대학교 농과대학 농생물학과

¹국립중앙과학관 자연사연구실²동국전문대학 약용식품학과

꼭지매미충(*Macrosteles striifrons*)은 각종 농작물을 가해하고 식물병원균을 매개하며 동부아시아 지역에 널리 분포하고 있으나, 종내변이는 물론 근사종과의 형태적 유사성이 심한 주요해충 중의 하나이다.

본 조사는 동부아시아 6개 지역에서 선발된 꼭지매미충의 수컷 8개체군을 대상으로 총 23개 정량형질에 대해 각 주요지역 계통간 이소적 특성, 기주식물 및 계절 등의 요인에 따른 동소적 특성, 우열도 및 그 상호관련성을 알아보기 위해 전자영상 미세측정방식과 고급 다변량 전산통계 분석법에 의한 종내 수리분류를 실시하였다.

그 결과, 지역별 평균분리도가 최저 90% 이상으로 매우 높았고, 기주별 및 계절별 등에 따른 종내 개체군간 평균분리도는 80% 이상으로 나타났다.

검색어 : 계량형태, 수리분류, 판별분석, 매미목, 매미충과, 꼭지매미충, 동부아시아.

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